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Glyphosate Affects Seed Composition in Glyphosate-Resistant Soybean

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The cultivation of glyphosate-resistant (GR) soybeans has continuously increased worldwide in recent years mainly due to the importance of glyphosate in current weed management systems. However, not much has been done to understand eventual effects of glyphosate application on GR soybean physiology, especially those related to seed composition with potential effects on human health. Two experiments were conducted to evaluate the effects of glyphosate application on GR soybeans compared with its near-isogenic non-GR parental lines. Results of the first experiment showed that glyphosate application resulted in significant decreases in shoot nutrient concentrations, photosynthetic parameters, and biomass production. Similar trends were observed for the second experiment, although glyphosate application significantly altered seed nutrient concentrations and polyunsaturated fatty acid percentages. Glyphosate resulted in significant decreases in polyunsaturated linoleic acid (18:2n-6) (2.3% decrease) and linolenic acid (18:3n-3) (9.6% decrease) and a significant increase in monounsaturated fatty acids 17:1n-7 (30.3% increase) and 18:1n-7 (25% increase). The combined observations of decreased photosynthetic parameters and low nutrient availability in glyphosate-treated plants may explain potential adverse effects of glyphosate in GR soybeans.

KEYWORDS: Glyphosate-resistant soybean (*Glycine max* L.); glyphosate; seed composition; fatty acids; nutrient status; photosynthesis

INTRODUCTION

Soybean is one of the major crops cultivated worldwide, and soybean seed quality is determined by protein, oil, and mineral contents (*1*). However, many farmers have noticed that some glyphosate-resistant (GR) soybean varieties are sensitive to water stress and are often visually injured after glyphosate application (*2*,*3*). Others have reported that the nutritional status of GR soybeans is strongly affected by glyphosate (*4*).

Glyphosate is a nonselective, broad-spectrum metal-chelating herbicide that is translocated throughout the plant to actively growing tissues where it inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway. This pathway is responsible for the biosynthesis of aromatic amino acids, plant defense mechanisms, and phenolic compounds (*5*).

Previous studies have shown that the basic composition of GR soybean seed is equivalent to that of conventional soybeans (*6*). However, recent research has demonstrated that α-linolenic acid (LNA, 18:3n-3) levels decreased and oleic acid increased under high glyphosate application rates (*7*) and that glyphosate drift resulted in a significant decrease in the iron (Fe) concentration in seeds (*1*).

LNA, an omega-3 (n-3) fatty acid (FA), and linoleic acid (LA, 18:2n-6), an omega-6 (n-6) FA, are considered strictly essential because humans cannot synthesize these FAs. Soybean and freshwater fish are good sources of LNA, which is metabolized by the same sequential desaturation and elongation enzyme systems, resulting in the production of long-chain polyunsaturated fatty acids (PUFA) of the omega-3 series (*8*). The importance of PUFA, particularly n-3 fatty acids, in human nutrition is widely recognized (*9*). n-3 PUFA have been associated with numerous benefits to human health.

The ratio between n-6 and n-3 (n-6:n-3) fatty acids in the diet is a critical factor influencing cardiovascular health (*10*); low ratios from 5 to 10 have been recommended by several health departments around the world (*11*). Some researchers believe that this ratio has moved from 20 to 30 in Western diets in the past few years, a value considered extremely high since the ideal would be between 1 and 2 (*12*).

Despite the widespread adoption of GR technology and the importance of glyphosate in weed control in worldwide cropping systems, few studies have been conducted to understand eventual

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effects of glyphosate application on GR soybean physiology, especially those related to the seed composition with effects on human health. The objective of this research was to evaluate specifically tissue and seed nutrient concentrations and seed fatty acid composition in GR soybean. To study the possible effect of the glyphosate gene (EPSPS) where both GR soybean and non-GR soybean had similar genetic background, both BRS 242 GR and its near-isogenic non-GR parental line Embrapa 58 with no EPSPS gene were used. Photosynthesis parameters and biomass production were also studied for possible relations with nutrient concentrations and seed composition constituents.

MATERIALS AND METHODS

Soil and Cultivation Practices. Two experiments were carried out in a greenhouse experiment equipped with an evaporative cooling system (25−35 °C: 20−22 °C day/night) under natural daylight conditions at the State University of Maringá, PR. The first experiment was conducted between October 2007 and February 2008 and the second experiment between November 2008 and March 2009 (location: 23° 25′ S, 51° 57′ W).

Plants were grown in polyethylene pots filled with 5.0 dm^{-3} of soil collected from the A horizon (0−20 cm) and sieved to pass through a 5 mesh screen. The Typic Hapludox soil contained 75% clay, 16% sand, 9% silt, pH_{CaCl_2} 5.70; Al, 0.0 $cmol_c \cdot dm^{-3}$; Ca, 8.71 $cmol_c \cdot dm^{-3}$; Mg, 3.22 $cmol_c \cdot dm^{-3}$; K, 1.13 $cmol_c \cdot dm^{-3}$; P, 18.01 $mg \cdot dm^{-3}$; S, 9.61 $mg \cdot dm^{-3}$; Fe, 64.43 $mg \cdot dm^{-3}$; Zn, 13.87 $mg \cdot dm^{-3}$; Cu, 25.61 $mg \cdot dm^{-3}$; Mn, 148.58 $mg \cdot dm^{-3}$; and C_{org}, 9.45 $g \cdot dm^{-3}$. Soil physicochemical properties were determined according to procedures established by Embrapa (*13*).

Seeds of soybean (*Glycine max* L.) near-isogenic non-GR parental line cv. Embrapa 58 and GR soybean cv. BRS 242 GR, both early maturity cultivars, were treated with 200 mL 100 kg^{-1} seed of a mixture of 200 g L^{-1} carboxim, 200 g L^{-1} thiram (concentrated suspension of systemic and contact fungicide), 13.5 g L^{-1} cobalt, and 135 g L^{-1} molybdenum. Seeds were then inoculated with a commercial rate of 300 mL 100 kg^{-1} seeds of a culture of *Bradyrhizobium elkanii*, strains SEMIA 587 and SEMIA 5019, at a concentration of 5×10^9 rhizobia g^{-1}. Four seeds per pot were sown at 3 cm depth and thinned to one plant per pot at V1 growth stage.

Glyphosate Treatments. Commercially formulated isopropylamine salts of glyphosate (480 g ae L^{-1}) were applied to cv. BRS 242 GR at V4 growth stage (30 days after sowing, DAS) at a rate of 1200 g ae ha^{-1}, and results were compared to a control without glyphosate and to the near-isogenic non-GR parental line (cv. Embrapa 58) under no glyphosate application. A total of 12 replicates per treatment were used. Six replicates were taken at R1 growth stage for shoot and root biomass. The other six replicates were kept growing and harvested at R8 for seed analysis.

Plants at the V4 growth stage were sprayed at 7:00 a.m. with glyphosate at 190 L ha^{-1} outside the greenhouse using a backpack sprayer with SF110.02 nozzles under a constant pressure of 2 kg cm^{-2} CO_2. Environmental conditions during glyphosate application were air temperature between 25 and 29 °C, relative humidity between 80% and 89%, wet soil, and wind speed between 5 and 10 km h^{-1}. The sprayed solution did not cause runoff from leaves. After glyphosate application, plants were returned to the greenhouse and irrigated the following day to keep the soil moist and to ensure absorption of the herbicide. The pots were irrigated daily to keep the soil moist and were hand-weeded as needed.

Photosynthetic Parameters. At R1 growth stage (46 and 54 DAS; 16 and 24 days after glyphosate application, for the first and second experiment, respectively), photosynthetic rate (*A*) was evaluated using an infrared gas analyzer (IRGA), ADC (Analytical Development Co. Ltd., Hoddesdon, U.K.) model LCpro+. The evaluations were between 7:00 and 11:00 a.m., choosing the last fully expanded trifolium (diagnostic leaf) of plants in each pot. The records were taken by automatic time-logging equipment with two measures of 3 min for each diagnostic leaf.

The SPAD reading were taken using a Minolta SPAD-502 m at the terminal leaflet of the diagnostic leaf, which measured absorption at 650 and 940 nm wavelengths and estimated the chlorophyll concentration (*14*). The SPAD sensor was placed randomly on leaf mesophyll tissue only, with veins avoided. Two leaves were chosen per plant in the pot, and measurements were immediately taken per leaf and averaged to provide a single SPAD unit.

Nutrient Concentration Measurements and Yield Parameters. In the first and second experiment, after collecting photosynthetic parameters at R1 growth stage, the diagnostic leaf was collected from each plant to determine its macro- and micronutrient concentration. However, in the second experiment, after these assessments, half of 12 replicates were collected to take the shoot and root dry biomass. Thus, aerial parts of soybean plants were clipped close to the soil, and roots were gently washed under running water to remove the soil. All harvested materials were then packed in paper bags and dried in a circulating air oven at 65−70 °C until a constant weight was achieved. Shoot and root biomass were determined by weighing plant parts.

The other 6 of the 12 replicates in the second experiment were maintained until R8 growth stage (136 DAS), when plants were harvested. Plants were evaluated for height, number of pods and seeds per plant, and weight of pods and seeds per plant. The mineral composition (P, K, Ca, Mg, S, Zn, Mn, Fe, Cu) of seed was determined by complete perchloric nitric digestion (6:1); B concentration was obtained after dry digestion (*15*). All elements, except N, were measured using an AES Perkin-Elmer ICP (inductively coupled plasma) spectrophotometer. Nitrogen was determined using sulfuric acid digestion and measured by the micro-Kjeldahl method (*16*).

Fatty Acid Determination. Seed total lipids were determined according to Bligh and Dyer (*17*). The fatty acid methyl esters (FAME) were prepared according to Joseph and Ackman (*18*). FAME were analyzed on a Shimadzu 14-A (Kyoto, Japan) gas chromatograph (GC) equipped with a flame ionization detector (FID) and a fused silica capillary column CP-Select CB-FAME (100 m × 0.25 mm id, 0.25 μm film thickness; Varian, EUA). The operation parameters were as follows: detector temperature, 240 °C; injection temperature, 240 °C; column temperature, from 180 to 280 °C at 5 °C/min and 280 °C final holding time of 35 min; carrier gas, hydrogen at 1.2 mL/min; makeup, nitrogen gas at 30 mL/min; split injection at 1:100 ratio. An amount of 1.0 μL of each sample and peak areas were determined in a GC-300 computing integrator (GC Instruments, Brazil), and FAME were identified by comparison with known retention times of standards obtained from Sigma (USA). Fatty acid identification was based on authentic reference standards (Sigma, USA) and equivalent chain-length values (ECL) (*19*).

Statistical Analyses. For both experiments, treatments were distributed in a randomized block design; however, the first experiment was conducted with four replicates, and all parameters were analyzed at R1 growth stage (46 DAS). The second experiment included 12 replicates from which nondestructive parameters (*A*, SPAD) were collected at the R1 stage (54 DAS), whereas destructive parameters (macro- and microconcentration on diagnostic leaf and shoot and root biomass) were analyzed from half of the replicates. The remaining replicates were sampled at the R8 stage (138 DAS), when height, number, and mass of pods and seeds per plant and fatty acids and mineral concentration in seeds were analyzed.

All data were subjected to analysis of variance. For the first experiment, comparison was done using the Scott Knott test at $p \leq 0.05$. For the second experiment, the LSD test was conducted using SISVAR variance analysis software (*20*), and $p \leq 0.05$ was used as the level significance. The significant probability of each variable response in the second experiment is presented in this study.

RESULTS AND DISCUSSION

Photosynthetic Parameters. In the first experiment at R1 growth stage (46 DAS), comparisons between BRS 242 GR without glyphosate with its near-isogenic non-GR parental isoline (cv. Embrapa 58) also without glyphosate revealed no differences in photosynthetic rate (A) or chlorophyll (SPAD) readings in both experiments. However, photosynthetic rates decreased in cv. BRS 242 GR + glyphosate compared with its near-isogenic line in both experiments (**Table 1**). No consistency in SPAD readings was shown in the two experiments. The inconsistency of some nutrient concentrations across the 2 years was expected because of seasonal variability, especially in temperature and radiation. The seasonal effect could be reflected under greenhouse conditions. Previous studies reported that exposure of plants of different maturity groups to a single or to

Table 1. Photosynthetic Rate (*A*), SPAD, Macro- and Micronutrient Concentrations of the Diagnostic Leaf, and Shoot and Root Dry Biomass at R1 Growth Stage in GR Soybean (BRS 242 GR) with and without Glyphosate and Its Near-Isogenic Non-GR Parental Lines (Embrapa 58) without Glyphosate

treatments	*A* (μmol of CO_2 m^{-2} s^{-1})	SPAD units	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	S (g kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})	shoot (g $plant^{-1}$)	root (g $plant^{-1}$)
							2007/2008[a]								
BRS 242 GR	16.49 a	35.7 a	33.70 a	2.14 b	23.11 b	10.65 b	3.70 b	1.83 b	44.18 b	232.73 b	168.00 b	22.11 a	34.18 b	12.62 a	7.24 a
BRS 242 GR + glyphosate	14.42 b	31.5 a	29.55 a	1.76 c	20.47 c	8.28 c	2.89 c	1.48 c	37.64 c	163.67 c	127.15 c	13.55 b	28.53 c	9.62 b	5.08 b
Embrapa 58	20.97 a	37.3 a	32.62 a	3.16 a	27.04 a	13.61 a	5.19 a	2.56 a	72.67 a	270.27 a	219.28 a	24.21 a	49.79 a	13.54 a	4.48 b
							2008/2009[b]								
BRS 242 GR	12.49 a	31.6 a	35.41 a	2.18 a	13.65 a	13.06 b	4.87 a	1.07 a	54.66 a	104.80 a	74.18 a	9.45 a	54.70 a	16.74 a	6.15 a
BRS 242 GR + glyphosate	10.13 b	27.4 b	34.71 a	1.68 b	10.28 a	8.80 c	4.19 b	1.14 a	47.30 b	78.83 b	78.78 a	7.94 b	54.28 a	10.06 b	4.98 a
Embrapa 58	12.81 a	33.4 a	33.68 a	1.88 ab	8.42 a	15.77 a	4.77 ab	0.97 a	49.68 ab	99.86 ab	60.07 a	7.28 b	46.50 a	15.22 a	6.37 a
DMS	1.00	2.46	5.50	0.454	5.55	2.45	0.56	0.21	6.37	23.94	19.09	0.83	8.80	5.03	1.73
CV (%)	4.48	3.91	7.96	10.04	16.51	9.77	6.37	9.75	6.40	19.51	11.76	5.32	7.25	21.56	14.85
Pr > Fc	0.01	0.01	ns	0.05	ns	0.01	0.03	ns	0.05	0.05	ns	0.01	ns	0.05	ns

[a] Data represent the average of four independent replicates. [b] Data represent the average of 12 independent replicates. Means within a column followed by the same letter are not significantly different at the 5% level as determined by LSD test.

sequential application of glyphosate decreased chlorophyll concentrations compared with the nonexposed plants (*4*). In our study, photosynthetic parameters *A* and SPAD were severely affected by glyphosate in GR soybeans; however, there were no differences between the nontreated GR soybeans and its respective near-isogenic non-GR parental lines (**Table 1**).

Cultivar BRS 242 GR treated with glyphosate exhibited chlorotic symptoms compared with the nontreated plants. The chlorotic symptoms may be related to decreased photosynthetic rates as a result of direct damage of glyphosate to chlorophyll (*2*, *21*) or immobilization of Mg and Mn (due to glyphosate−cation nutrient complexes) required for chlorophyll formation and photosynthesis, respectively. The accumulation of the main glyphosate metabolite, aminomethylphosphonic acid (AMPA), in the glyphosate-treated plants may also be a source of injury and chlorosis (*2*, *22*, *23*). Visual injuries are likely to happen in GR soybeans after glyphosate application. They are usually considered nonpersistent as the yellow flashing tends to disappear within the first 2 weeks after herbicide application (*24*). It must be noted here that effects of glyphosate on chlorophyll and photosynthesis parameters do not necessary lead to yield differences unless these parameters are measured over the entire growing season under field conditions.

Mineral Status in Diagnostic Leaf and Seeds. All tissue macro- and micronutrients, except nitrogen, were reduced by glyphosate in the first experiment compared with the nontreated glyphosate GR soybean and its near-isogenic non-GR parental line (cv. Embrapa 58) (**Table 1**). However, for the second experiment, only Ca concentrations were reduced by glyphosate in cv. BRS 242 GR (**Table 1**). Comparing BRS 242 GR with BRS 242 + glyphosate, glyphosate resulted a significant decrease in concentrations of P, Ca, Mg, Zn, and Mn. For example, comparing BRS 242 GR, the percentage decrease in tissue nutrient concentrations in BRS 242 GR + glyphosate in 2007/2008 was 17.8% for P, 22.3% for Ca, 14.8% for Zn, 24.3% for Mn, 38.7% for Cu, 23.8% g $plant^{-1}$ for shoot biomass, and 30% for root biomass. Similar trends were observed in 2008/2009. Also, glyphosate application significantly altered seed nutrient concentrations. For example, comparing BRS 242 GR, the percentage decrease in seed nutrient concentrations in BRS 242 GR + glyphosate was 15.7% for P, 18.3% for Ca, 22.1% for Mn, and 12.8% for Cu. Compared with BRS 242 GR + glyphosate, the parental line with no glyphosate application showed greater concentrations in shoot P, Ca, Cu, seed Ca, and monounsaturated fatty acids but lower concentrations of polyunsaturated fatty acids.

These results demonstrated that glyphosate or one of its metabolites apparently remained active in soybean through the R1 growth stage or later, indicated by the decrease in mineral concentrations in leaf tissue. This suggests that cultivar BRS 242 GR may be inefficient in nutrient uptake and translocation or was unable to rapidly recover from potential chelating effects of glyphosate applied at V4 growth stage (*25*, *26*).

Other field observations in Brazil and the North Central United States have reported that frequent applications of glyphosate induce Fe, Zn, and Mn deficiencies in GR soybean (*27*). Several studies have concluded that glyphosate affects micronutrient nutrition of plants, which has been correlated with an ability of glyphosate to form insoluble glyphosate−metal complexes (*4*, *25*, *28*). According to Eker et al. (*26*), after absorption of glyphosate into the plants, the uptake and transport of cationic micronutrients may be inhibited by the formation of poorly soluble glyphosate−metal complexes within plant tissues. This also could explain the lower micronutrient concentration by BRS 242 GR + glyphosate when compared to BRS 242 GR without glyphosate or to Embrapa 48 (**Table 1**).

Bellaloui et al. (*1*) found that glyphosate drift rates resulted in a significant decrease in the Fe concentration in soybean leaves and seeds. This same reduction of Fe concentration in leaves was noticed in our first experiment; however, for the second experiment decreases in Fe concentration in leaves and seeds were not detected and may be due to genotype differences and seasonal effects of temperature and radiation. In fact, other seed nutrients were decreased by glyphosate, including P, Ca, Mn, Zn, and Cu (**Table 2**), which were accompanied by the decreased nutrient concentrations found in diagnostic leaves (**Table 1**). Similar decreases in Ca, Mg, Fe, and Mn in seeds of non-GR soybean subjected to sublethal concentrations of glyphosate were reported by Cakmak et al. (*29*), who suggested that these nutrients were immobilized by glyphosate thereby interfering with uptake and translocation by the plant. Duke et al. (*23*) noticed that glyphosate was translocated into metabolic sinks such as seeds, where both glyphosate and AMPA were detected, suggesting the possibility that glyphosate and AMPA may indirectly affect nutrient uptake and translocation. Because these compounds

Table 2. Macro- and Micronutrient Concentration in Seed at R8 Growth Stage in GR Soybean (BRS 242 GR) with and without Glyphosate and Its Near-Isogenic Non-GR Parental Lines (Embrapa 58) without Glyphosate[a]

treatments	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	S (g kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})
BRS 242 GR	60.00 a	26.02 a	15.98 a	3.28 a	3.25 a	3.31 a	65.90 a	32.50 a	35.80 a	8.86 a	16.00 a
BRS 242 GR + glyphosate	60.66 a	21.94 b	15.76 a	2.68 b	2.96 a	3.37 a	52.76 ab	25.33 b	34.20 b	7.73 b	16.88 a
Embrapa 58	59.33 a	27.58 a	16.13 a	3.38 a	3.20 a	3.89 a	49.80 b	26.56 b	35.13 ab	8.06 a	22.97 a
DMS	3.58	2.55	0.80	0.57	0.51	1.69	15.18	5.80	1.19	0.71	5.20
CV (%)	2.99	5.07	2.52	9.18	8.18	24.10	13.54	12.77	5.99	4.33	19.37
Pr > Fc	ns	0.01	ns	0.04	ns	ns	0.05	0.05	0.05	0.02	ns

[a] Data represent the average of six independent replicates. Means within a column followed by the same letter are not significantly different at the 5% level as determined by LSD test.

Table 3. Height and Yield Parameters at R8 Growth Stage in GR Soybean (BRS 242 GR) with and without Glyphosate and Its Near-Isogenic Non-GR Parental Lines (Embrapa 58) without Glyphosate[a]

treatments	height (cm)	no. of pods (units plant^{-1})	no. of seeds (units plant^{-1})	wt of pods (g plant^{-1})	wt of seeds (g plant^{-1})
BRS 242 GR	73.33 a	88.00 a	170.66 a	40.33 a	24.22 a
BRS 242 GR + glyphosate	66.66 a	60.00 a	127.66 b	33.15 a	21.03 b
Embrapa 58	64.66 a	76.00 a	161.33 a	37.33 a	24.13 a
DMS	10.12	30.80	24.22	4.20	3.09
CV (%)	7.43	20.65	7.91	5.70	6.70
Pr > Fc	ns	ns	0.01	ns	0.05

[a] Data represent the average of six independent replicates. Means within a column followed by the same letter are not significantly different at the 5% level as determined by LSD test.

can remain within the plant until complete physiological maturity (*30*), the decrease in macro- and micronutrient concentrations in leaf tissues and seed noticed in our experiment may be due to the potential chelating effects to forming glyphosate−cation nutrient complexes.

Shoot and Root Biomass. At R1 stage, shoot dry biomass was significantly reduced by glyphosate use in both experiments. However, root biomass decreased but not significantly (**Table 1**). The effect of glyphosate on shoot was more pronounced, possibly because BRS 242 GR is an early maturity group cultivar that has a short time to recover from glyphosate injury. The combined effects of maturity and genotype on the process of recovery from glyphosate cannot be excluded because effects of maturity and genotype could not be separated in this experiment. Negative effects on biomass accumulation are probably due to additive effects of decreased photosynthesis and reduced nutrient concentration (**Table 1**). By applying glyphosate rates much higher than those in this work at 1680 g ae ha^{-1} in Reddy et al. (*31*) and at 6300 g ae ha^{-1} in King et al. (*32*), other authors have also found decreased shoot and root dry weights of GR soybean after glyphosate use under greenhouse conditions. These findings also are supported by Bott et al. (*28*), who noted that the commercial application of glyphosate to a GR soybean cultivar significantly inhibited root biomass and root elongation.

The potential for GR soybean injury from glyphosate has been reported previously and has been partially associated with AMPA formed from glyphosate degradation, since the extent of injuries appeared to be largely dependent on AMPA concentrations formed within the plant (*33*). This metabolite is known to be phytotoxic to GR soybean, resulting in reduced chlorophyll content and shoot fresh weight (*2*). In a recent study, Zobiole et al. (*4*) found that glyphosate application either sequentially (600 + 600 g ae ha^{-1}) or as single dose (1200 g ae ha^{-1}) decreased shoot and root biomass in GR soybean compared with near-isogenic, nontreated non-GR soybean or nontreated GR soybean. It is possible that the way glyphosate was applied (single or sequential) could have contributed to the decreased shoot and root biomass.

Height and Yield Parameters. At R8 growth stage, plant height and number and weight of pods per plant were not affected by glyphosate; however, the number and weight of seeds per plant were reduced by 25% and 13%, respectively, with glyphosate (**Table 3**). No differences were found between cultivars with no glyphosate treatments. Since cv. BRS 242 GR is a cultivar of an early maturity group, the severe injury may be due to the shorter period of detoxification of glyphosate or one of its metabolites such as AMPA (*2*, *23*).

Fatty Acids. A total of 15 FA that comprised the seed total lipid contents were identified (**Table 4**). Major individual seed FA included linoleic acid (LA, 18:2n-6), oleic acid (OA, 18:1n-9), and α-linolenic acid (LNA, 18:3n-3) for all samples. Glyphosate treatment increased the percentages of monounsaturated fatty acid 17:1n-7, 18:1n-9 (OA), and 18:1n-7 percentages, which are not essential to the human diet, but decreased the percentages of two important polyunsaturated fatty acids (PUFAS), LA and LNA. A previous report also noted that glyphosate reduced LNA and increased OA (*7*). The lowest concentrations of LA and LNA were found in glyphosate-treated BRS 242 GR compared with nontreated BRS 242 GR and the near-isogenic non-GR parental line (cv. Embrapa 58) (**Table 4**). LA and LNA are essential FA, are not synthesized by humans, yet have important functions in human brain activity (*34*). In fish and monogastric animals, LA and LNA are metabolized by the same sequential desaturation and elongation enzyme systems, which results in the production of omega-3 (n-3) and omega-6 (n-6) FA, such as eicosapentaenoic acid, 20:5n-3 (EPA), docosahexaenoic acid, 2:6n-3 (DHA), and araquidonic acid, 20:4n-6 (AA) (*35*).

In general, there was no difference between BRS 242 GR without glyphosate and its near-isogenic non-GR parental line (cv. Embrapa 58) in saturated fatty acids (SFA), PUFA, monounsaturated fatty acid (MUFA), total n-6 fatty acids, omega-6: omega-3 (n-6:n-3) ratio, and PUFA:SFA ratio, except for n-6:n-3 ratio (**Table 5**). However glyphosate-treated BRS 242 GR had an

Table 4. Effect of Glyphosate on Fatty Acid Composition in Seeds from GR Soybean (BRS 242 GR) with and without Glyphosate and Its Near-Isogenic Non-GR Parental Lines (Embrapa 58) without Glyphosate[a]

	fatty acid composition (%)														
treatments	14:0	16:0	16:1n-9	17:0	17:1n-7	18:0	18:1n-9	18:1n-7	18:2n-6	20:0	18:3n-6	18:3n-3	21:0	22:0	24:0
BRS 242 GR	0.073 a	10.52 a	0.096 a	0.076 b	0.033 b	3.42 b	16.48 b	1.53 b	60.08 a	0.026 a	0.283 a	8.33 a	0.013 b	0.033 a	0.28 b
BRS 242 GR + glyphosate	0.073 a	10.41 a	0.093 a	0.073 b	0.043 a	3.45 b	18.62 a	2.11 a	58.53 b	0.026 a	0.256 ab	7.53 b	0.026 a	0.030 a	0.35 a
Embrapa 58	0.076 a	10.52 a	0.080 b	0.086 a	0.030 b	3.90 a	13.05 c	2.10 a	60.93 a	0.030 a	0.230 b	8.04 a	0.020 ab	0.033 a	0.16 c
DMS	0.011	0.68	0.009	0.003	0.009	0.25	1.95	0.201	1.483	0.009	0.035	0.502	0.009	0.009	0.049
CV (%)	7.76	3.25	5.24	7.32	13.26	3.58	6.11	7.11	1.24	16.97	6.99	3.35	23.57	14.63	9.31
Pr > Fc	ns	ns	0.01	0.05	0.03	0.01	0.01	0.01	0.02	ns	0.03	0.02	0.03	ns	0.01

[a] Data represent the average of six independent replicates. Means within a column followed by the same letter are not significantly different at the 5% level as determined by LSD test.

Table 5. Effect of Glyphosate on Fatty Acid Composition in Seeds from GR Soybean (BRS 242 GR) with and without Glyphosate and Its Near-Isogenic Non-GR Parental Lines (Embrapa 58) without Glyphosate[a]

treatments	SFA (%)	PUFA (%)	MUFA (%)	n-6 (%)	n-3 (%)	n-6:n-3 ratio	PUFA:SFA ratio
BRS 242 GR	14.45 a	69.70 a	16.67 b	60.37 a	8.33 a	7.25 c	4.82 a
BRS 242 GR + glyphosate	14.44 a	66.32 b	20.89 a	58.79 b	7.53 b	7.81 a	4.59 a
Embrapa 58	14.84 a	69.21 a	15.29 b	61.17 a	8.04 a	7.60 b	4.66 a
DMS	0.909	1.747	1.882	1.477	0.502	0.143	0.232
CV (%)	3.12	1.28	5.35	1.23	3.35	2.94	2.50
Pr > Fc	ns	0.01	0.01	0.02	0.02	0.05	ns

[a] Data represent the average of six independent replicates. Means within a column followed by the same letter are not significantly different at the 5% level as determined by LSD test. Abbreviations: SFA = saturated fatty acids; PUFA = polyunsaturated fatty acids; MUFA = monounsaturated fatty acid; n-6 = sum of polyunsaturated fat acids with double or triple bonds on carbon 6; n-3 = sum of polyunsaturated fat acids with double or triple bonds on carbon 6.

increased n-6/n-3 ratio (7.81) relative to BRS 242 GR without glyphosate (7.25), which is an important human nutrition value where a low ratio is considered most beneficial in a healthy diet and is recommended by the health care community (*11*).

Since glyphosate is partially metabolized to AMPA in soybean and both compounds are detected in metabolic sinks including seeds (*23*, *30*), AMPA and/or glyphosate may directly or indirectly affect FA desaturase activities or *de novo* synthesis of desaturase enzymes, affecting FA profiling and content. Negative effects of glyphosate on photosynthesis and mineral absorption could lead to an imbalance in C:N ratio, affecting FA profiling and PUFA production. Mechanisms of glyphosate effects on photosynthesis or nutrient uptake and translocation and how these affect PUFA are still not understood.

The present study demonstrated that near-isogenic non-GR parental lines and GR soybean plants without glyphosate treatment exhibited higher photosynthesis rates and functional chlorophyll and generally higher concentrations of macro- and micronutrients in leaves and seeds compared with GR soybeans receiving glyphosate. Omega-3 and omega-6 fatty acids, comprising the main group of PUFA in seeds, were also affected by glyphosate treatment of GR soybean. It may be concluded that glyphosate can alter soybean seed polyunsaturated fatty acid and seed mineral concentrations. Further studies are needed to understand the mechanisms of glyphosate effects on seed composition and mineral nutrition. Understanding these mechanisms is important for improving current glyphosate-based weed management systems that will not interfere with adequate seed FA composition and mineral nutrition qualities.

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